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TECHNICAL MANUSCRIPT 353

PRIMARY AND SECONDARY ANTIBODY RESPONSES OF CHICKENS TO PASTEURELLA TULARENSIS

John E. Nutter

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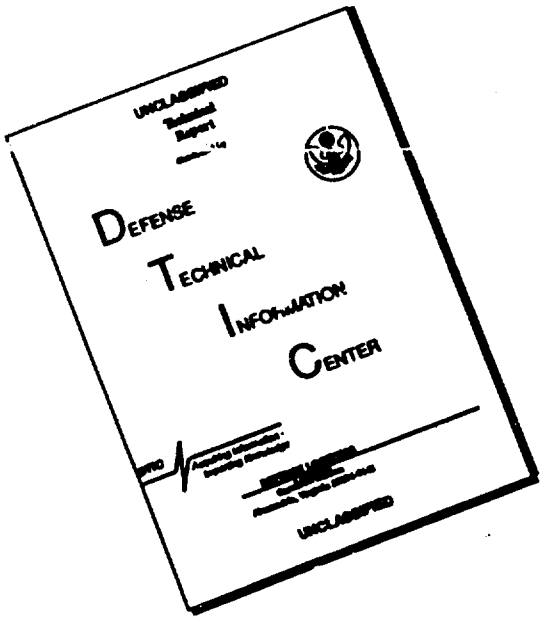

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DEPARTMENT OF THE ARMY
Fort Detrick
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TECHNICAL MANUSCRIPT 353

PRIMARY AND SECONDARY ANTIBODY RESPONSES OF CHICKENS TO *PASTEURELLA TULARENSIS*

John E. Nutter

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1B63301D165

January 1967

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

PRIMARY AND SECONDARY ANTIBODY RESPONSES OF CHICKENS TO PASTEURELLA TULARENSIS

ABSTRACT

The antibody responses of chickens to killed or viable Pasteurella tularensis were determined. Three preparations were employed: viable organisms of the live vaccine strain LVS, viable cells of strain SCHU S4 (highly virulent for most laboratory animals but not for chickens), or killed strain SCHU S4. Primary responses were initiated by the intravenous administration of 10^9 live or killed organisms. Secondary responses were induced by revaccination of each animal with the same preparation 28 days after the vaccine was first administered. Antibody was measured by bacterial agglutination, passive hemagglutination, and agar-gel precipitin techniques. All three methods provided data indicating that the viable SCHU S4 vaccine was superior to either the viable LVS vaccine or the killed SCHU S4 preparation. During the primary response highest levels of antibody were observed on the 12th day following vaccination; titers declined rapidly thereafter. Revaccination induced an anamnestic response only when viable LVS or killed SCHU S4 vaccines were employed. In no case did the secondary response to any of the three vaccines exceed appreciably the primary response to the viable SCHU S4 vaccine.

The chicken has been used to produce antisera against a variety of soluble and particulate antigens ranging from bovine serum albumin to the bacteriophage ϕX 174. In general, this species responds to vaccination with a more rapid production of antibodies than do mammalian hosts. There is, however, a lack of information regarding the use of the chicken for the production of antibacterial antibodies against highly virulent bacterial pathogens. Yager* has reported that the chicken produced high titers of anti-Pasteurella tularensis antibodies within 9 days after vaccination, but his study did not include an evaluation of the temporal synthesis. The purpose of this study was to determine the course of antibody production by chickens to killed or viable P. tularensis vaccines.

* Yager, R.H.; Spertzel, R.O.; Jaeger, R.F.; Tigertt, W.D. 1960. Domestic fowl: Source of high titer P. tularensis serum for the fluorescent antibody technic. Proc. Soc. Exp. Biol. Med. 105:651-654.

Both the primary and secondary responses were investigated. Antibody production was assayed by the bacterial agglutination, passive hemagglutination, and agar-gel precipitin techniques.

Two strains of P. tularensis were employed for vaccination, SCHU S4 (highly virulent for most laboratory animals but not for chickens) and live vaccine strain LVS. Each of these strains was employed as a viable preparation; in addition, a third vaccine was prepared by killing a SCHU S4 suspension with a solution of phenol and merthiolate.

White Rock chickens of both sexes were used and each animal received 1×10^9 to 2×10^9 bacteria of the appropriate vaccine intravenously. The secondary response was initiated on the 28th day, when each animal was revaccinated with the same vaccine employed for the primary response.

The results of the bacterial agglutination tests during the primary response are shown in Figure 1. Each point is the mean value from ten individual determinations. Throughout the primary response animals that received the viable SCHU S4 vaccine had the highest agglutinin titers. The highest mean titer (1:1,792) occurred 12 days after vaccination. On the 9th, 12th, and 15th days the titers of the chickens vaccinated with the viable SCHU S4 vaccine were significantly higher ($P \leq 0.05$) than those that had received either the killed SCHU S4 or the viable LVS preparation. Except for the values obtained on day 21, the titers of animals vaccinated with viable SCHU S4 remained significantly higher for the remainder of the primary response than those of the animals that had received the killed vaccine.

Secondary response agglutinin titers of animals that had received a second intravenous injection of the same antigen employed for the primary response are presented in Figure 2. Animals that received killed SCHU S4 or live LVS showed appreciable increases in agglutinin titers as early as 3 days after revaccination. A smaller increase in titer occurred 6 days after revaccination with viable SCHU S4. The highest sustained secondary agglutinin response was elicited by the administration of the viable LVS vaccine. Revaccination with either the LVS or the killed SCHU S4 vaccines clearly elicited an anamnestic response, but the response to the viable SCHU S4 vaccine was poorer than observed following primary vaccination.

For the passive hemagglutination study, the polysaccharide antigen was adsorbed to sheep erythrocytes. The primary hemagglutinin response is shown in Figure 3. In general, results were similar to those obtained by the bacterial agglutination technique. Peak titers were observed 12 days after administration of the vaccine but subsequently decreased rapidly. Of particular interest is the finding that viable SCHU S4 produced the highest titers, killed SCHU S4 vaccine the lowest, and LVS intermediate titers. These differences in titer were particularly marked at the 12th and 15th days, when the animals vaccinated with the viable SCHU S4 preparation had titers of 1:10,240, which were approximately fourfold higher than those vaccinated with either of the other preparations.

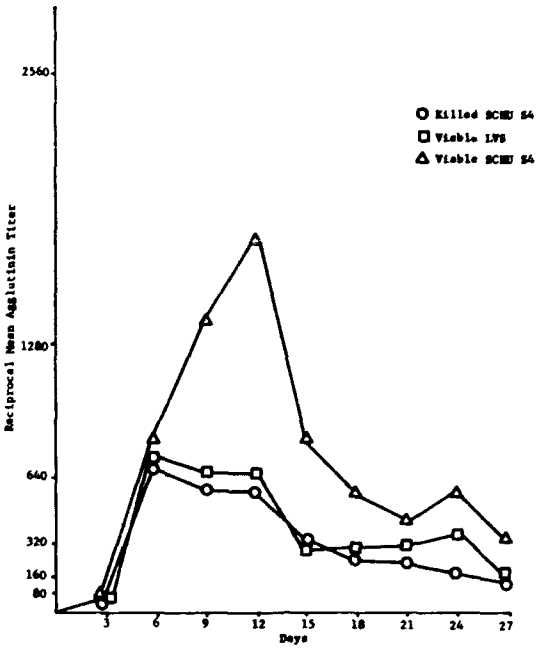

Figure 1. Agglutinin Response of Chickens Following Intravenous Administration of P. tularensis Live or Killed Vaccine.

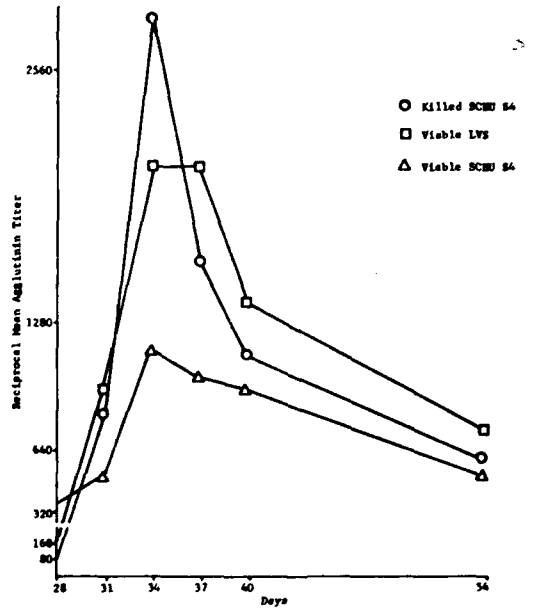

Figure 2. Agglutinin Response of Chickens Following a Second Intravenous Administration (Revaccination on 28th Day) of P. tularensis Live or Killed Vaccine.

The hemagglutinin titers during the secondary response are presented in Figure 4. In each case, highest titers were obtained on the 34th day, which was 6 days after revaccination. Both the killed SCHU S4 and the viable LVS vaccines elicited higher titers than did the viable SCHU S4 organisms. Animals vaccinated with killed SCHU S4 or viable LVS underwent an anamnestic response; i.e., the secondary response antibody levels were higher than those of the primary response and were achieved sooner. The response of the chickens to revaccination with the viable SCHU S4 vaccine was not marked when compared with that for the other vaccines and could not be termed an anamnestic response. The peak antibody levels were not maintained but decreased rapidly, especially in animals vaccinated with the killed SCHU S4 or the viable LVS.

The agar-gel precipitin assay (Ouchterlony technique) of the response to the various vaccines is shown in Table 1. The primary response to killed SCHU S4 was poor and only one precipitin band was visible with the samples from days 3 to 12. The secondary response to this preparation was only slightly better, with two bands produced on the 34th day and only one band at all other periods.

TABLE 1. AGAR-GEL PRECIPITIN RESPONSE OF CHICKENS VACCINATED WITH P. TULARENSIS VACCINES

	Number of Bands Observed													
	Primary Response on Day									Secondary Response on Day				
Vaccine	3	6	9	12	15	18	21	24	27	31	34	37	40	54
Killed SCHU S4	0	1	1	1	0	0	0	0	0	1	2	1	1	1
Viable LVS	0	3	3	3	1	1	1	1	1	3	3	3	2	2
Viable SCHU S4	0	4	4	5	5	5	5	5	4	4	4	4	4	4

During the primary response of the animals to the viable LVS vaccine, three bands appeared on the 6th through the 12th days. Revaccination of these animals did not increase the number of bands or prolong the period of antibody production.

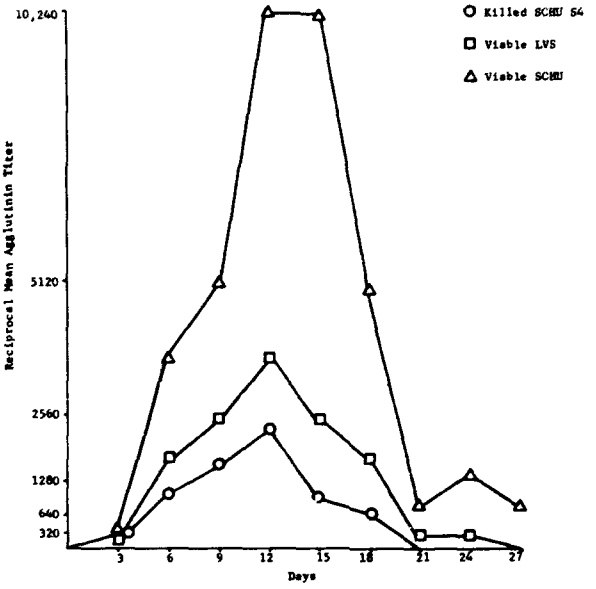

Figure 3. Passive Hemagglutinin Response of Chickens Following the Intravenous Administration of P. tularensis Live or Killed Vaccine.

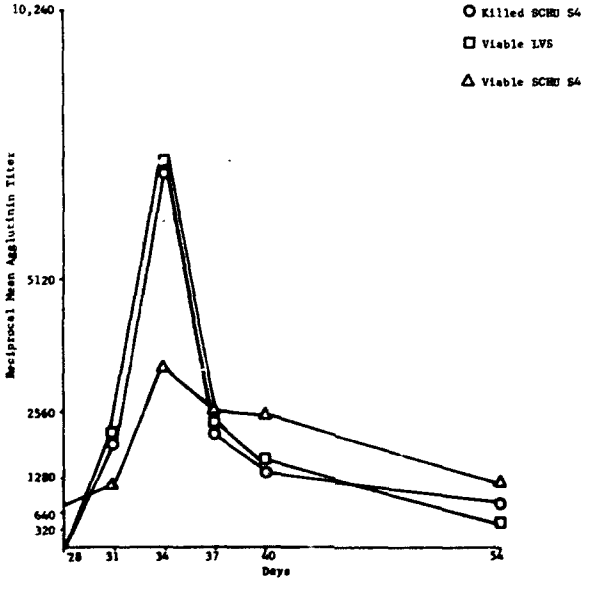

Figure 4. Passive Hemagglutinin Response of Chickens Following a Second Intravenous Administration (Revaccination on 28th Day) of P. tularensis Live or Killed Vaccine.

Vaccination of the chickens with viable SCHU S4 resulted in the best antibody production during the primary response. Five bands were observed from the 12th through the 24th day. Revaccination with this suspension did not result in an increased number of bands. The increased effectiveness of the viable SCHU S4 for precipitin production was also indicated by the rapidity with which the bands developed. Visible lines of precipitate were seen within 24 hours when the test was conducted with plasma from SCHU S4-vaccinated chickens. With serum from the other two groups of vaccines, 48 to 72 hours were required to produce visible precipitates.

In summary, the relative efficiencies of three different whole-cell P. tularensis vaccines for the production of antibodies in chickens were assessed by three techniques. All three methods gave similar results and data indicated that a viable vaccine of the highly virulent SCHU S4 strain was superior to a viable LVS vaccine or a killed preparation of SCHU S4 organisms. Maximal levels of antibody during the primary response were observed on the 12th day following vaccination. Revaccination induced an anamnestic response only when viable LVS or killed SCHU S4 vaccine was employed; in no case did the secondary response to any of the three vaccines exceed appreciably the primary response to the viable SCHU S4 vaccine.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)	2a REPORT SECURITY CLASSIFICATION
DEPARTMENT OF THE ARMY Fort Detrick, Frederick, Maryland, 21701	Unclassified
	2b GROUP

3 REPORT TITLE

PRIMARY AND SECONDARY ANTIBODY RESPONSES OF CHICKENS TO PASTEURELLA TULARENSIS

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Nutter, John E.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
January 1967	11	1

8a CONTRACT OR GRANT NO.	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO. 1B63301D165	Technical Manuscript 353
c	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

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13 ABSTRACT

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14. Key Words

*Pasteurella tularensis	*Chickens	Live vaccines
*Vaccines	Pasteurella tularensis LVS	Killed vaccines
*Antibodies	Pasteurella tularensis SCHU S4	

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